program. The annealing temperature of the three-step PCR program should be between 56°C and 64°C. 3) Extension time: It is recommended to use two-step PCR and set the extension time as 1 minute. To increase amplification efficiency, increase the extension time, or try three-step PCR.

3. Three-step PCR program:

Step	Temperature	Time	Cycles
Initialization	95°C	10 min[1)]	1
Denaturation	95°C	10 sec	35-40
Annealing	56-64°C[2)]	30 sec	
Extension	72°C	32 sec[3)]	
Melting curve analysis[4)]			

Note: 1) The hot-start enzyme used in this product must be pre-denatured at 95°C for 10 minutes to activate the enzyme. 2) If good amplification efficiency cannot be achieved, lower the annealing temperature appropriately. If there is non-specific reaction, increase the annealing temperature. 3) To increase amplification efficiency, increase extension time appropriately. 4) This program uses ABI7900 qPCR machine as an example. The melting curve analysis should be set according to the procedure recommended by qPCR instrument used.

2× UltraSYBR Mixture (High ROX)

Catalog Number:

CW2602S (1 ml)
CW2602M (5 ml)
CW2602L (25 ml)

Storage Condition:

-20°C

Kit Components:

Component	CW2602S (1 ml)	CW2602M (5 ml)	CW2602L (25 ml)
2× UltraSYBR Mixture (High ROX)	1 ml	5× 1 ml	25× 1 ml
ddH_2O	1 ml	5× 1 ml	25× 1 ml

Product Introduction:

The UltraSYBR Mixture (High ROX) is a premixed system for real-time fluorescence quantitative PCR (SYBR Green I), and the concentration is 2x. It contains GoldStar Taq DNA Polymerase, PCR Buffer, dNTPs, SYBR Green I Fluorescent Dye, Mg^{2+} and High ROX as reference dye. The operation is simple and convenient. This product is mainly used for the detection of genomic DNA target sequences and cDNA target sequences after RNA reverse transcription.

This product contains the fluorescent dye SYBR Green I which can bind with all double-strand DNA, so that the product can be used for the detection of different target sequences without the need for the synthesis of specific labeled probes. The GoldStar Taq DNA Polymerase in the mixture is a chemically-modified, new efficient hot-start enzyme that does not have polymerase activity at room temperature which prevents non-specific amplification efficiently, and it is activated by incubation at 95°C for 10 minutes. The combination of a unique PCR buffer system and a hot-start enzyme effectively inhibits non-specific PCR amplification and significantly increases the amplification efficiency of PCR.

The ROX dye contained can correct the fluorescence signal error between the wells of the quantitative PCR instrument. The amount of ROX in this kit is low, and it is suitable for quantitative PCR instruments which require high ROX for signal correction, such as ABI Prism7000/7300/7700/7900, Eppendorf, ABI Step One/Step One Plus etc.

Features:

This product uses a new high-performance hot-start enzyme (GoldStar Taq DNA Polymerase) and a unique PCR buffer system. This product significantly improves the PCR amplification efficiency, sensitivity, and specificity.

Precautions:

1. Mix gently before use, avoid foaming, and use after brief centrifugation.
2. This product contains SYBR Green I fluorescent dye and ROX dye. Avoid strong light irradiation during the storage.
3. Avoid repeated freezing and thawing. Repeated freezing and thawing may comprise product performance.
4. This product cannot be used for qPCR using probes.

Protocol:

The following protocol is an example of conventional PCR reaction. The actual protocol should be optimized based on the template, primer structure and the size of the amplicon.

1. PCR reaction system:

Reagent	50 μl	Final Conc.
2× UltraSYBR Mixture (High ROX)	25 μl	1×
Forward Primer, 10 μM	1 μl	0.2 μM[1)]
Reverse Primer, 10 μM	1 μl	0.2 μM[1)]
DNA template	2 μl[2)]	< 5 ng/μl
ddH_2O	Up to 50 μl[3)]	

Note: 1) Usually 0.2 μM of primer concentration gives better results, and the final concentration of primers should be between 0.1 and 1.0 μM. 2) Usually the amount of DNA template is 10-100 ng for genomic DNA or 1-10 ng for cDNA. 3) The recommended reaction volume is 50 μl, and the reaction volume can also be scaled up or down according to actual experimental requirements.

2. PCR reaction program:

This program uses ABI7900 qPCR machine as an example. If a good result cannot be obtained due to the low Tm of the primers, try a three-step PCR program.

Step	Temperature	Time	Cycles
Initialization	95°C	10 min[1)]	1
Denaturation Annealing/Extension[2)]	95°C 60°C	15 sec 1 min	35-40
Melting curve analysis[3)]			

Note: 1) The hot-start enzyme used in this product must be pre-denatured at 95°C for 10 minutes to activate the enzyme. 2) The annealing temperature should be between 60-64°C. If there is non-specific reaction, increase the annealing temperature. 3) This program uses ABI7900 qPCR machine as an example. The melting curve analysis should be set according to the procedure recommended by qPCR instrument used.

Optimization of reaction conditions:

1. The experimental system with high reaction specificity and high amplification efficiency should be as the following conditions: 1) High specificity: no non-specific amplification such as primer dimers for negative control; No other amplification beyond the target fragment. 2) High amplification efficiency: Low Ct value; Amplification efficiency of PCR is high, close to the theoretical value of 100%.
2. Methods of optimization of reaction conditions: 1) Primer concentration: Usually 0.2 μ M of primer concentration gives better results, and the final concentration of primers should be between 0.1 and1.0 μM. To increase the specificity of the reaction, decrease the concentration of the primer; To increase the amplification efficiency, increase the concentration of the primer. 2) Annealing temperature: It is recommended to use two-step PCR, and set the annealing temperature as 60°C. To increase the specificity, increase the annealing temperature, which should be between 60-64°C. If a good result cannot be obtained due to the low Tm of the primers, try a three-step PCR